

I CLAIM:

1. A method for identifying unculturable microorganisms comprising the steps of:

isolating at least one bacterial cell from an environmental sample comprising a plurality of microorganisms;

amplifying at least one DNA fragment from said at least one bacterial cell;

cloning said at least one DNA fragment into at least one *E. coli* vector;

sequencing said at least one DNA fragment, resulting in identification of at least one DNA sequence; and

comparing said at least one DNA sequence with existing DNA databases, resulting in identification of said at least one DNA sequence as one of an unculturable microorganism and a known microorganism.

2. A method in accordance with Claim 1, wherein said at least one DNA fragment is amplified by a polymeric chain reaction (PCR) using at least one universal primer.

3. A method in accordance with Claim 2, wherein said universal primer is an oligonucleotide of arbitrary sequence.

4. A method in accordance with Claim 3, wherein said oligonucleotide comprises in a range of about 8 bp to about 20 bp.

5. A method in accordance with Claim 2, wherein said at least one universal primer is one of a high-GC content primer and a high-AT content primer.

6. A method in accordance with Claim 2, wherein a pair of said at least one universal primer comprises two primers selected from the group consisting of high-GC content primers, high-AT content primers and mixtures thereof.

7. A method in accordance with Claim 2, wherein said at least one universal primer comprises a random mixture of oligonucleotides having a common length and differing in DNA sequence.

8. A method in accordance with Claim 1 further comprising identifying at least one said DNA sequence suitable for use as a species-specific DNA sequence.

9. A method in accordance with Claim 1, wherein said at least one bacterial cell is isolated from said environmental sample with a micromanipulator.

10. A method in accordance with Claim 1, wherein said at least one bacterial cell is isolated from said environmental sample using flow cytometry.

11. A method in accordance with Claim 8, wherein at least one hybridization probe/DNA chip array is prepared using said species-specific DNA probe.

12. A method in accordance with Claim 8, wherein at least one PCR primer pair suitable for targeting at least one unique said DNA sequence is prepared using said species-specific DNA sequence.

13. A method in accordance with Claim 11, wherein said species-specific DNA probe comprises in a range of about 20 bp to about 2000 bp.

14. A method in accordance with Claim 12, wherein said PCR primers used to amplify said species-specific DNA sequence comprises in a range of about 20 bp to about 50 bp.

15. A method in accordance with Claim 1, wherein a plurality of said DNA fragments of various lengths are derived from multiple loci throughout a chromosome of said unculturable microorganism.

16. A method in accordance with Claim 6, wherein additional said environmental samples are subjected to at least one condition, at least one of total DNA and/or total RNA is obtained from said additional said environmental samples, and said species-specific DNA probe is used in methods selected from the group consisting of PCR, RT-PCR and microarray hybridization/gene expression, resulting in generation of data concerning responses of said unculturable microorganisms to said at least one condition.

17. A method in accordance with Claim 1, wherein at least one fluorescent dye is used to differentially stained said plurality of microorganisms which are subsequently processed by flow cytometry and cell sorting to produce at least two sub-populations that differ in terms of at least one of species composition and species relative abundance from said environmental sample.

18. A method in accordance with Claim 17, wherein at least one of said sub-populations is subjected to dilution culture experiments utilizing a plurality of bacterial growth media, resulting in growth of at least one species of previously unculturable microorganism.

19. A method in accordance with Claim 17, wherein at least one of said sub-populations is subjected to genetic analysis to detect and analyze 16S rRNA sequences to obtain improved data regarding the biodiversity of said environmental sample.

20. A method in accordance with Claim 17, wherein at least one of said sub-populations is used to prepare at least one genomic library.

21. A method in accordance with Claim 17, wherein at least one of said sub-populations is further processed by FACS to obtain at least one individual bacterial cell.

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